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MicroRNAs and LPS: Developing a Relationship in the Neonatal Gut

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Upon birth, the intestine converts from a sterile environment to a home for commensal microorganisms. How immune homeostasis is maintained during this transition is not well understood. Here, Chassin et al. (2010) demonstrate that microRNA-146a regulates the responsiveness of intestinal epithelial cells during microbial colonization of the neonatal intestine.

The task of distinguishing friend from foe is particularly complex in the gut due to the presence of commensal microbiota. While most sites of immune surveillance are sterile, which enables straightforward microbial detection, cells in the gut coexist with 10^{13} microbial neighbors. Consequently, the gut has evolved mechanisms that ignore (or at least tolerate) commensal neighbors yet respond strongly to the occasional pathogenic intruder. A considerable body of work has focused on understanding how this complex relationship is managed in adults, but an equally important issue is how this balance is initially established during the first colonization of the neonatal intestine. In this issue of *Cell Host & Microbe*, Hornef and colleagues describe a regulatory pathway that keeps the peace in the neonatal gut during this transition (Chassin et al., 2010).

How homeostasis is preserved in the gut is a quickly evolving area of research. Multiple immunoregulatory mechanisms have been implicated in maintaining tolerance to commensals, including T regulatory cells, immunosuppressive cytokines, and negative regulators of innate immune signaling (Hooper and Macpherson, 2010). Despite recent progress in these areas, it remains poorly understood how the cells in closest contact with gut microbes, intestinal epithelial cells (IECs), avoid overt responses to commensals (Hooper and Macpherson, 2010). While this regulation likely involves multiple pathways, one proposed mechanism is that IECs do not express innate receptors or sequester receptors on the basolateral membrane to avoid responses to commensals. However, it has previously been demonstrated that IECs express Toll-like receptor 4 (TLR4) and respond to lipopolysaccharide (LPS) (Lotz et al., 2006), suggesting that additional regulatory mechanisms must exist. An alternative model argues that most microbes are physically separated from IECs by the mucosal lining within the gut.

Most of our understanding of gut homeostasis comes from studies in adult mice. A largely unexplored area of research is how the IECs respond to the initial wave of bacteria during intestinal colonization. Hornef and colleagues examine the host response to this initial colonization by comparing vaginally delivered mice with mice born by Caesarean section (Chassin et al., 2010). Mice delivered vaginally were exposed to the mother's microbial flora and quickly became unresponsive to LPS by downregulating the essential signal transduction protein IRAK1. Mice that were delivered via Caesarean section were not exposed to microbial flora, did not downregulate IRAK1, and remained responsive to LPS.

The authors go on to show that the regulation of IRAK1 is mediated by the microRNA miR-146a, which is itself upregulated in response to LPS via TLR4. By artificially changing the levels of miR-146a, Chassin et al. (2010) demonstrate that upregulation of miR-146a in the developing gut is essential to protect the neonatal intestine from mucosal damage after the introduction of a large bolus of LPS or bacteria (Figure 1). This tolerance persists until the third week of life, when increased IEC proliferation depletes intracellular LPS, restoring IRAK1 protein expression.

The change in IRAK1 levels after the first 3 weeks of postnatal development is quite surprising. LPS is still very much present in the gut 3 weeks after birth, so what can explain the change in the regulation of IRAK1? One possibility is that the miR-146a repression of IRAK1 does not operate in adult IECs. This possibility seems unlikely, though, as downregulation of IRAK1 (as well as other TLR signaling components) by miR-146a occurs in many cells types and seems to be a general anti-inflammatory mechanism in adult mice (Nahid et al., 2009; Taganov et al., 2006). Another possible explanation for the regained responsiveness of IECs to LPS is that they are no longer constantly exposed to LPS from the gut. It has been suggested that the mucosal barrier within the gut prevents bacteria from being able to interact with the epithelial lining. This compartmentalization is largely mediated by mucins. Mice that lack Muc2, a glycoprotein that forms the mucosal barrier in the gut, are unable to generate the mucosal lining necessary to physically separate gut microbes from IECs and develop colitis several weeks after birth (Johansson et al., 2008). Additionally, studies with germ-free mice have shown that the physiology of the gut is influenced by the presence of microbes (Lesher et al., 1964; Stappenbeck et al., 2002). Thus, it seems possible that initial microbial stimulation within the neonatal gut leads to further maturation and eventual separation of IECs from commensal microbes.

Interestingly, Chassin et al. (2010) also demonstrate that tolerant IECs are not completely nonresponsive to innate stimuli. While tolerized IECs no longer produced inflammatory cytokines in response to LPS, they did induce a distinct set of genes that helped maintain gut homeostasis. Surprisingly, induction of

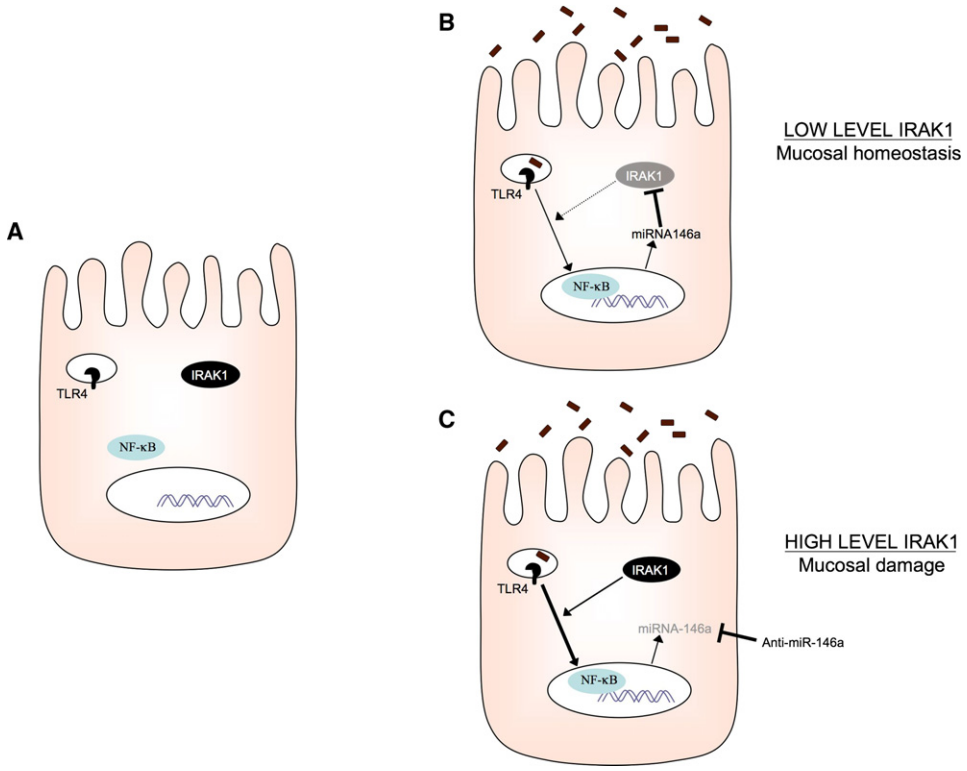

Figure 1. Micro-RNA-146a Protects the Neonatal Gut from LPS-Induced Mucosal Damage
(A) Before birth, the gut is sterile, and IECs do not encounter bacteria.
(B) After natural birth, the gut is colonized with a complex flora of bacteria. IECs are exposed to LPS and upregulate microRNA-146a (miRNA-146a). Upregulation of microRNA-146a leads to the degradation of IRAK1. When IRAK1 is expressed at low levels, TLR4 signaling promotes mucosal homeostasis.
(C) If microRNA-146a activity is inhibited, IRAK1 is not degraded upon TLR4 activation within IECs. High levels of IRAK1 in the postnatal gut leads to excessive cytokine production and mucosal damage when neonates are challenged with bacteria.

these genes required the same TLR4/IRAK1 signaling pathway that leads to the production of inflammatory cytokines in IECs. This bifurcation in the transcriptional response to LPS has been observed before in a different context: naive versus tolerized macrophages (Foster et al., 2007). While tolerized macrophages no longer induce most inflammatory genes in response to LPS, they still induce a set of genes involved in bacterial killing. This work and the current work by Chassin et al. ascribe these transcriptional differences to distinct but not mutually exclusive mechanistic properties—chromatin remodeling versus weak levels of signal transduction. It will be interesting to determine whether both pathways work in parallel to facilitate the lack of ignorance to LPS in tolerized cells.

Hornef and colleagues have developed a powerful system to investigate the initial interactions during colonization of the sterile neonatal gut. They have shown that miR-146a is necessary to protect mice from a potentially harmful inflammatory response to LPS in the postnatal gut. However, this particular regulatory pathway is surely only one of many processes that facilitate this initial interaction between host and microbe. Indeed, a recent analysis of mice lacking miR-146a reveals that these mice remain healthy during the first few weeks of life, suggesting that additional mechanisms must prevent responses to commensals in the absence of miR-146a (Lu et al., 2010). Further examination of the developing gut will surely provide additional insights into the regulatory mechanisms that make the gut such a welcoming place for the trillions of bacteria that call it home.

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